

**Cell Reports, Volume 30**

**Supplemental Information**

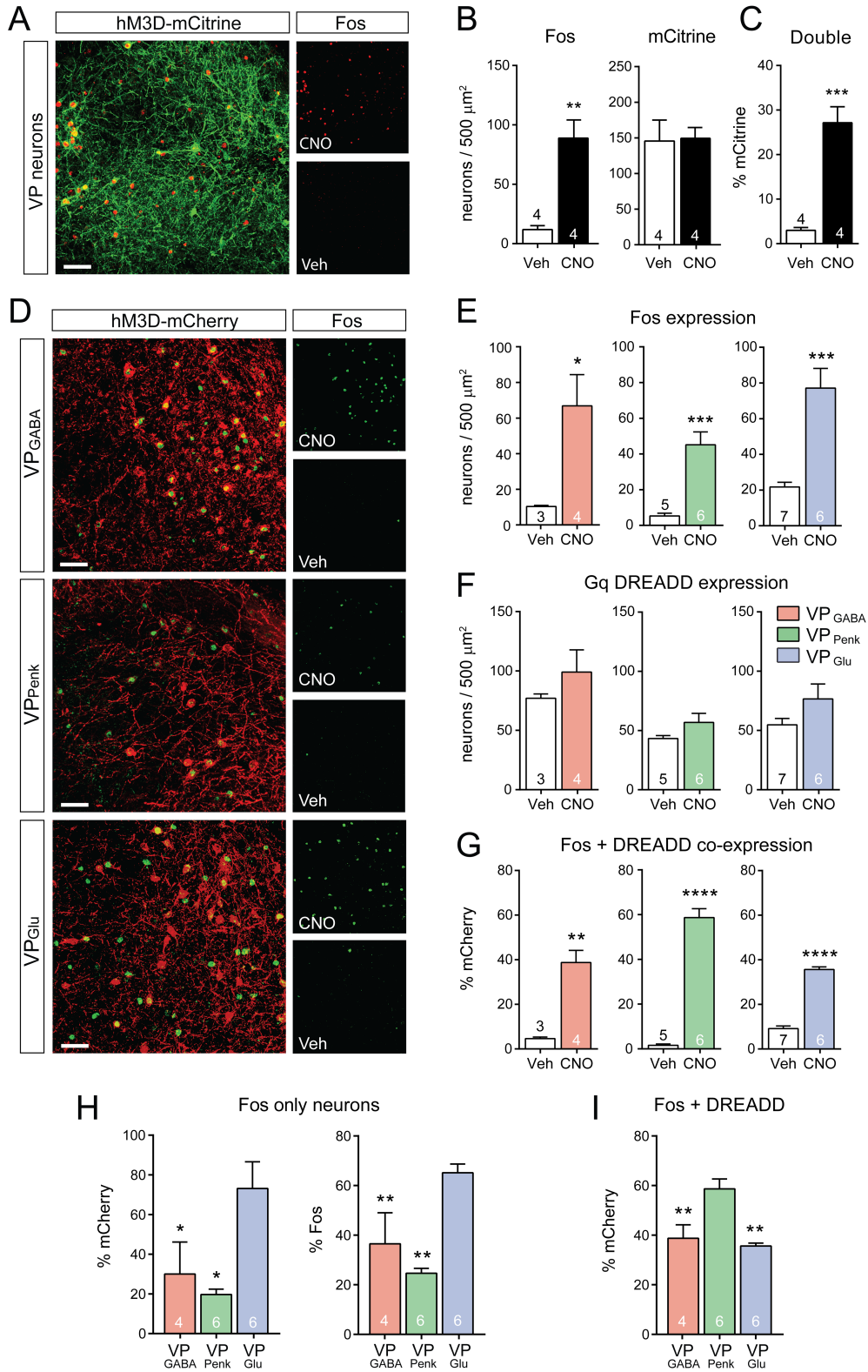
**Opposing Regulation of Cocaine Seeking**

**by Glutamate and GABA Neurons**

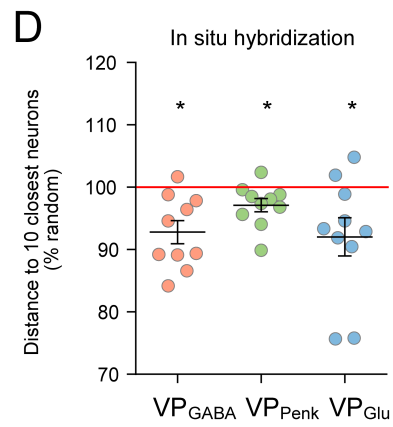
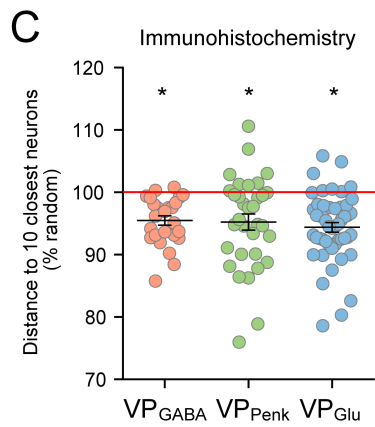
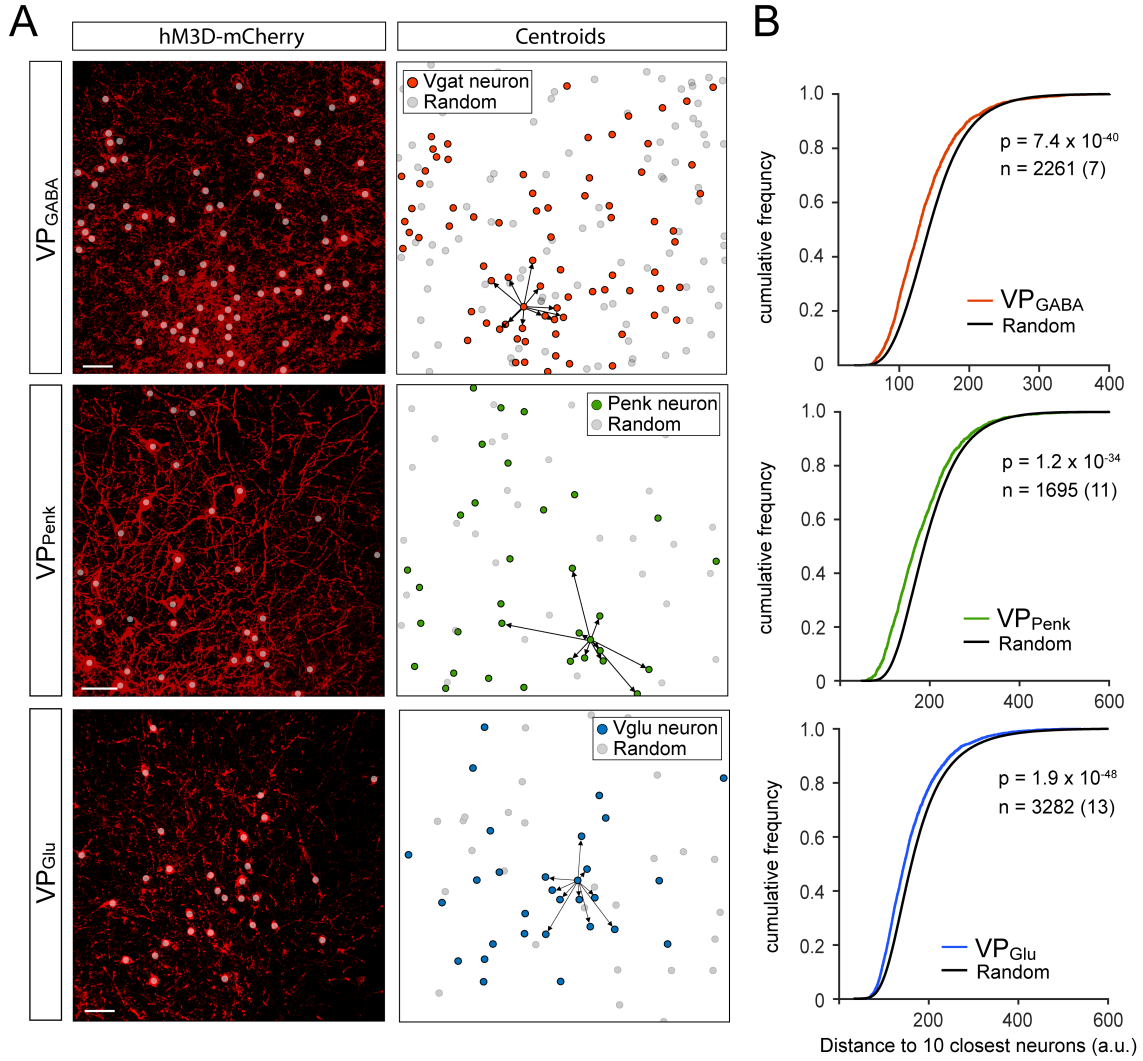
**in the Ventral Pallidum**

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**SUPPLEMENTAL INFORMATION**

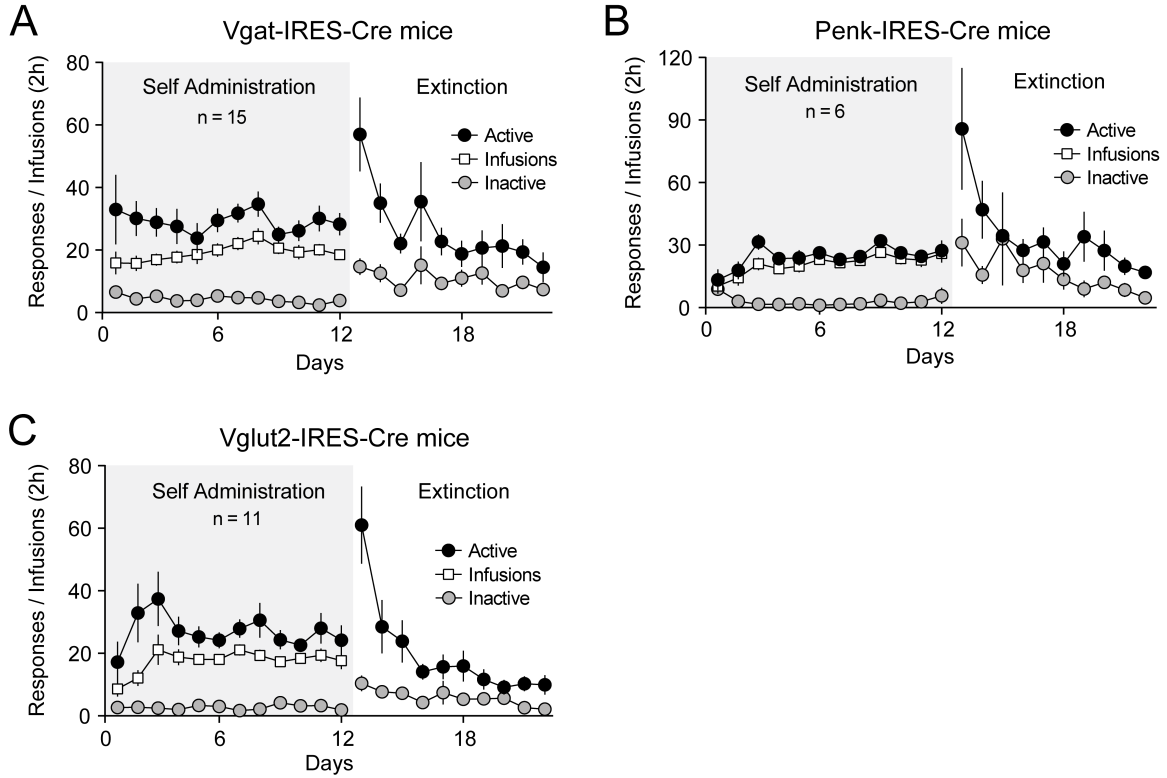


**Figure S1. Gq DREADD Activation by CNO Induces Fos in VP Neurons, Related to Figure 2 and 3.** (A) Representative micrograph showing that Fos expression induced by CNO co-localizes with Gq DREADD (mCitrine) expression in the VP. Inserts to the right show Fos expression following an injection of CNO (top) or vehicle (Veh, bottom). Bar= 50  $\mu$ m. (B) CNO significantly induces Fos in the VP of animals infected with Gq DREADD. (C) CNO induces Fos in Gq DREADD virus (mCitrine) expressing neurons. (D) Representative images showing Fos and Gq DREADD co-expression in VP<sub>GABA</sub>, VP<sub>Penk</sub> and VP<sub>Glu</sub>. Inserts on the right show Fos induction in an animal injected with CNO (top) or Veh (bottom). Bar= 50  $\mu$ m. (E) Gq DREADD activation significantly induced Fos in all VP neuronal subtypes. (F) Gq DREADD (mCherry) expression levels were similar between CNO and Veh treated mice. (G) CNO significantly induced Fos in virus infected neurons across all cell-type. (H) Non-virus infected neurons were Fos activated in significantly higher numbers following stimulation of VP<sub>Glu</sub> compared to VP<sub>GABA</sub> or VP<sub>Penk</sub> neurons (counts normalized to total virus expression or total Fos expression). (I) CNO induces Fos in a larger fraction of VP<sub>Penk</sub> compared to VP<sub>GABA</sub> or VP<sub>Glu</sub> neurons. n in bars = number of animals. Data represented as mean  $\pm$  SEM. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001 (for H comparing VP<sub>GABA</sub> and VP<sub>Penk</sub> to VP<sub>Glu</sub>, for I comparing VP<sub>GABA</sub> and VP<sub>Glu</sub> to VP<sub>Penk</sub>).

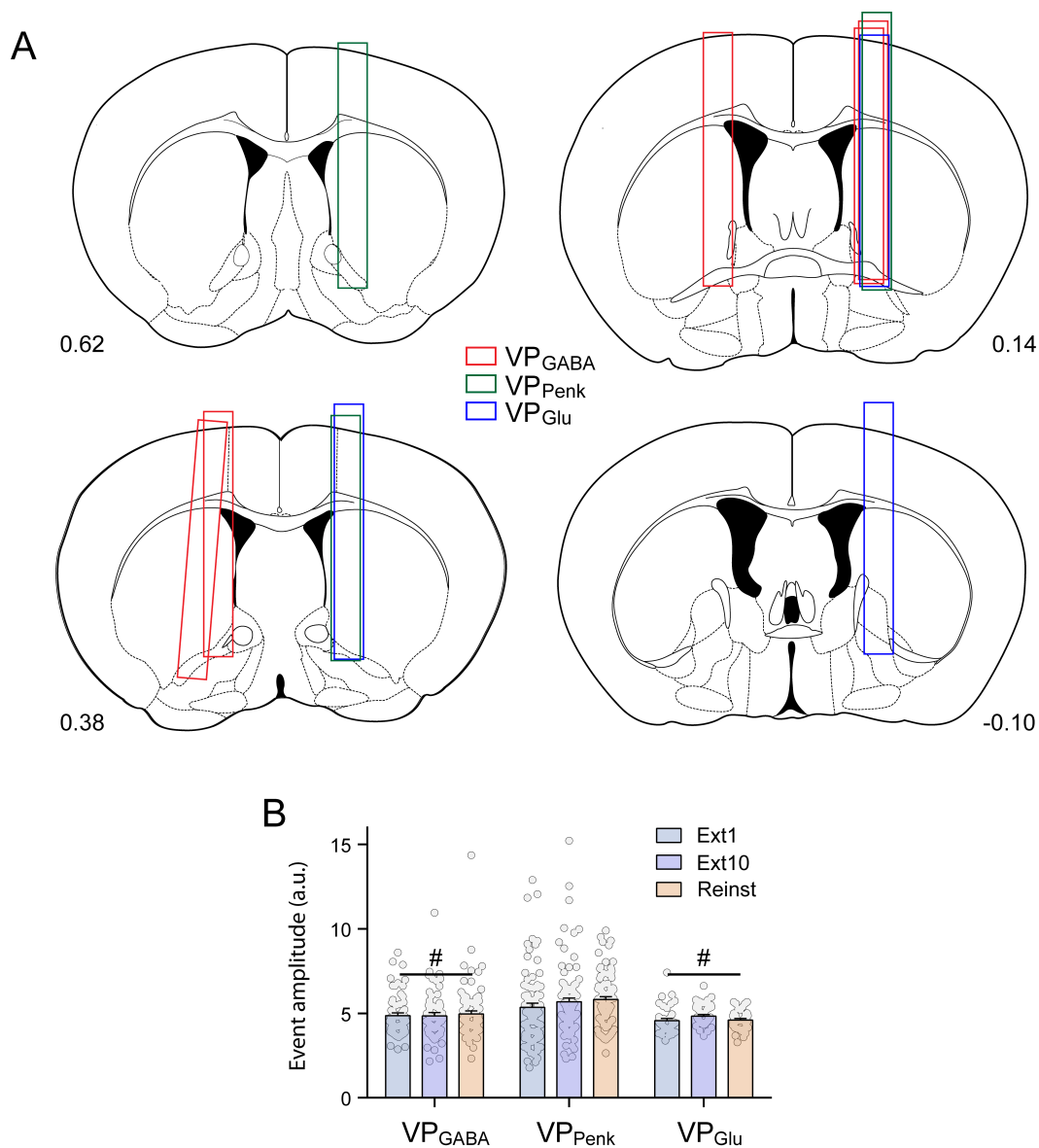




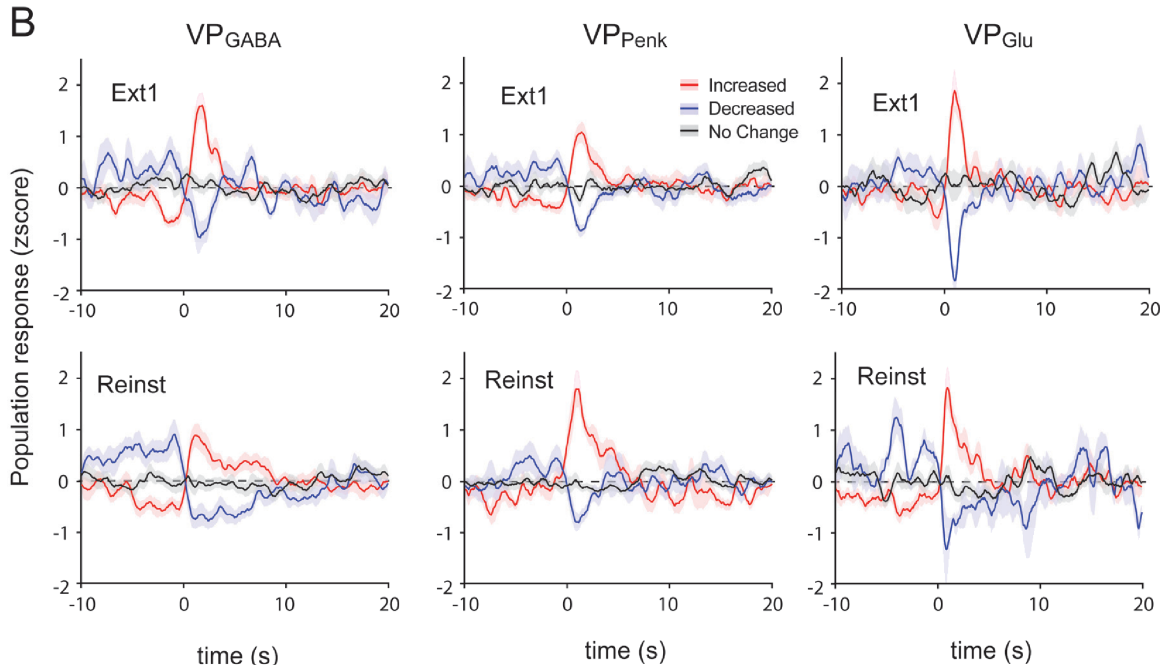
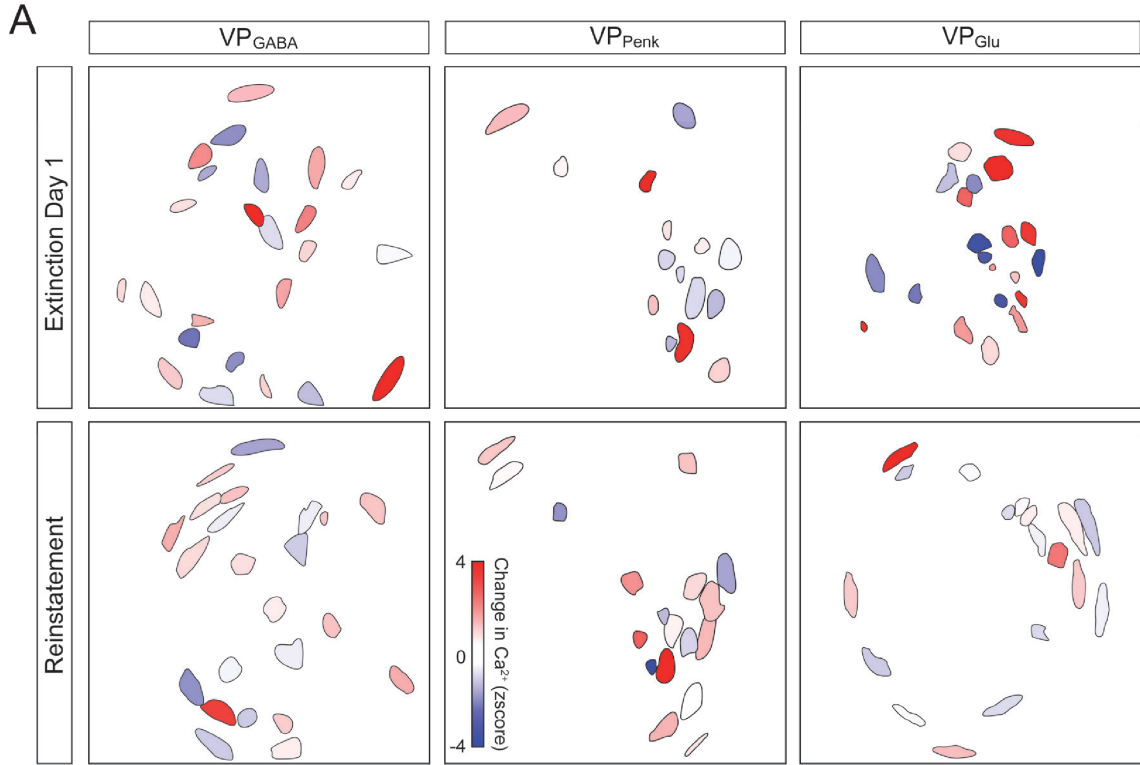
**Figure S2. VP Subpopulations are Organized in Clusters, Related to Figure 3.** (A) Left: micrographs showing mCherry expression in subpopulations of VP neurons with overlaid centroids for each cell (white dots). Right: Example centroid maps (color) for representative image stacks of each cell type combined with a randomly generated centroid map (grey) containing the same number of datapoints, as well as an example showing the 10 nearest neighbors of a single cell. (B) Cumulative frequency distribution for the distances of each VP cell to its 10 nearest neighbors, and the equivalent distance between random datapoints. VP subpopulations were significantly more clustered than random data (2-sample Kolmogorov-Smirnov test)  $n = \text{total cell\# over (mouse \#)}$  collected in 3-4 image stacks per animal. (C) K-nearest neighbor distance for all cells per image stack (normalized to the equivalent distance between random datapoints in that stack). VP neurons identified by mCherry expression (D) or RNA expression (E) were significantly more organized as clusters than random data (one-sample t-test). Error bars represent SEM. \*  $p < 0.05$



**Figure S3. Cocaine Self-administration and Extinction for Gq DREADD Experiment, Related to Figure 3.** (A) Vgat-IRES-Cre mice, (B) Penk-IRES-Cre mice, and (C) Vglut2-IRES-Cre lines acquired stable self-administration after 3d and extinguished operant responding in the absence of cocaine. Data represented as mean  $\pm$  SEM.



**Figure S4. GRIN Lens Placements and Ca<sup>2+</sup> Event Amplitudes, Related to Figure 4 and 5.** (A) Placements of GRIN lenses for recordings of the different VP subpopulations of neurons. Numbers represent anteroposterior coordinates relative to Bregma. (C) Ca<sup>2+</sup> event amplitude does not differ across conditions, but the Ca<sup>2+</sup> event amplitude of VP<sub>Penk</sub> neurons is significantly higher than that of VP<sub>Glu</sub> and VP<sub>GABA</sub> neurons. Data represented as mean ± SEM. # p < 0.05 compared to VP<sub>Penk</sub>.



**Figure S5. Populations of VP Neurons Subdivided by Increased or Decreased Activity Patterns, Related to Figure 5.** (A) Example cell maps of the distinct VP neurons isolated from  $\text{Ca}^{2+}$  videos, pseudocolored by activity profile showing neurons responding with increased or decreased activity during the first 2s following a nosepoke are spread heterogeneously throughout the VP. (B) Population responses for all cell types and conditions separated by neurons that show increased, decreased or unchanged  $\text{Ca}^{2+}$  activity 2s following a nosepoke. Data represented as mean (lines)  $\pm$  SEM (shading).